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Brassinosteroid biosynthesis and signalling in *Arabidopsis thaliana* and *Petunia hybrida*

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2011

document version

Publisher's PDF, also known as Version of record

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citation for published version (APA)

Verhoef, N. (2011). *Brassinosteroid biosynthesis and signalling in Arabidopsis thaliana and Petunia hybrida*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

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Summary

As the world population is growing continuously, food production needs to keep pace. Apart from improved fertilization and cultivation techniques, plant breeding greatly improved productivity.

One of the major abiotic stresses that affects productivity in the agricultural sector is soil salinity. Desertification, floods and unsuitable irrigation methods increase the amount of salt all over the world. Removal of salt from soil is costly and therefore it is economically important to find solutions that allow crops to grow in salty habitats. Currently two genetic approaches are used to improve salt stress tolerance in crops: 1) direct selection of existing plant varieties that are salt tolerant and cross them into cultivars and 2) the generation of transgenic plants to introduce novel genes or alter the level of expression of existing genes that play a role in salt stress tolerance.

The initial goal of my PhD research project was to investigate the role of the GSK3-like kinase GSK1 in salt stress signalling by using the model plant *Arabidopsis thaliana*. The *Arabidopsis* genome contains ten GSK3-like kinases that based on phylogenetic analysis can be classified into four subgroups. BIN2, a group II member, is the best-studied GSK3-like kinase and plays a negative role in the brassinosteroid (BR) signalling pathway.

Based on previous research by another group we expected that mutation of the *GSK1* gene would result in a plant more susceptible to salt stress. We considered this might be the same for two other GSK3-like kinases (*ASK ζ* and *BIN2*) as they are very similar to GSK1, all belonging to the group II GSK3-like kinases. In **Chapter 2**, we checked whether mutation of these genes altered the response of *Arabidopsis* towards salt. In contrast to prior research, we could not detect a role for *GSK1* in tolerance against Na⁺ stress, neither for the other group II members *ASK ζ* and *BIN2*. However, based on research by others, the involvement of not only group II GSK3-like kinases but also GSK3-like kinases of other subgroups in salt tolerance seems likely. Instead of playing a positive role in salt stress resistance, GSK3-like kinases seem to play a negative role. The potential role of GSK3-like kinases in salt stress signalling might be an indirect effect that originates from the role that we and others discovered for GSK1 as well as *ASK ζ* in BR signalling.

Brassinosteroids (BR) are steroid hormones that are essential for plant growth and development. **Chapter 1** reviews the current knowledge about BR biosynthesis, BR signalling and its involvement in various growth- and developmental processes such as cell

division and elongation, vascular differentiation, abiotic- and biotic stresses, photo-morphogenesis, germination, senescence and fertility.

BIN2 negatively regulates the BR signalling pathway by phosphorylating the transcription factors BES1 and BZR1. In **Chapter 2** we show that GSK1 as well as ASK ζ interact with the BES1/BZR1 transcription factor family. The fact that group II GSK3-like kinases have redundant roles in the BR signalling pathway was confirmed by studying several kinds of mutants.

In order to learn more about the mode of actions of GSK1 and the group II GSK3-like kinases in BR signalling and possibly in other signal transduction pathways we tried to purify protein complexes containing group II GSK3-like kinases via various Tandem Affinity Purification (TAP) methods which are described in **Chapter 3**. Till date, TAP purification was unsuccessful due to technical complications and maybe also because the TAP tagged versions of GSK3-like kinases seem to be biologically inactive.

BR biosynthesis and signalling has been extensively studied in Arabidopsis and to some extent in other plant species such as tomato, rice and *Catharanthus roseus*. Within our petunia collection we found several mutant families that resemble sterol/BR biosynthesis and signalling mutants and this tempted us to research whether the BR biosynthesis and signalling pathways are also conserved in petunia (**Chapter 4**). Cross-pollination between the mutant families revealed five different complementation groups. Four mutant groups clearly responded to spraying with the most biologically active BR, 24-epibrassinolide, by elongation of their internodes, petioles and leaves. Complementation of the mutant phenotype in these plants indicated that these are sterol/BR biosynthesis mutants. For one mutant group we could reveal that this mutant phenotype was caused by the insertion of a transposable *dTph1* element in the BR biosynthesis gene *CPD*. One mutant group did not respond to exogenous application of 24-epibrassinolide and therefore it was considered to be a BR signalling mutant. Interestingly, this family turned out to have a *dTph1* transposon insertion in a gene homologous to the BR receptor BRI1 from Arabidopsis.

With help of a PCR-based approach we succeeded to isolate a member of the BES1/BZR1 transcription factor family (PhBEH2) and a group II GSK3-like kinase (PSK9) from petunia. In a yeast two-hybrid experiment PhBEH2 interacted with another group II GSK3-like kinase (PSK8) and 14-3-3 proteins, supporting the idea that the BR signalling pathway is conserved among the plant kingdom. This was further supported by the fact that PSK8 could interact with members of the BES1/BZR1 family from Arabidopsis and BIN2 from Arabidopsis with PhBEH2 from petunia.

Interestingly PhBEH2 also interacted with proteins involved in gibberellin (GA), auxin (IAA) and abscisic acid (ABA) signalling and a protein that plays a role in the autonomous flowering pathway. These interactions reveal new mechanisms, not recognised from

Arabidopsis research that suggest extensive cross-talk between BR signalling and other (hormone) signalling pathways.

This thesis provides some knowledge about BR biosynthesis and signalling in Arabidopsis and petunia. For a long time BRs are applied in the agriculture and horticulture to increase crop yield and quality. With help of the acquired knowledge about the BR pathway, superior crops could be developed in the future with a desirable stature, high yield and good quality.